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Effects of elevated ground-level ozone on paddy soil bacterial community and assembly mechanisms across four years

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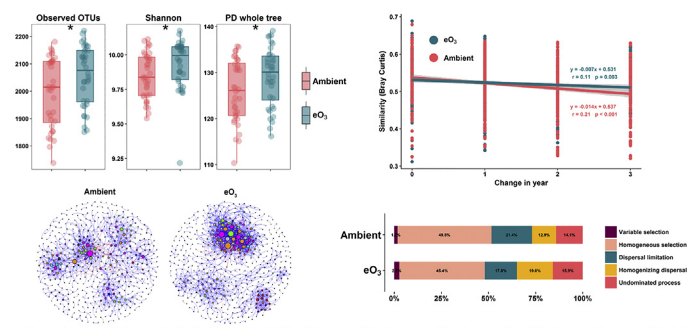
HIGHLIGHTS

- Temporal responses and assembly of paddy bacterial community to eO_3 were evaluated.
- eO_3 diversified and centralized the community due to microbial survival strategy.
- eO_3 slowed temporal turnover of community by damaging relationships with plants.
- eO_3 adversely influenced bacterial community and ecological functions they provided.

GRAPHICAL ABSTRACT



Elevated ground-level ozone led to the higher complexity of the co-occurrence network and alpha diversity of paddy bacterial community

Elevated ground-level ozone slowed the temporal turnover of bacterial community compositon by decreasing deterministic processes

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ABSTRACT

It is well known that elevated ground-level ozone (eO_3) poses a threat to the ecosystem. Little knowledge about the underground variables, especially on soil microorganisms, however, has been revealed. Such knowledge will tremendously help to advance our understanding of the correlation between ecosystems and climate change, as well as our ability to predict future trajectory. For this purpose, we have collected soil DNA samples (eO_3 vs. Ambient, each having 36 samples) over four years. Our results have verified the temporal responses and the underlying assembly mechanisms of the paddy bacterial community to eO_3. Contrary to the widespread consensus, it was found that eO_3 stimulated bacterial alpha diversities. The higher complexity and the centralization of the co-occurrence network of the bacterial community suggested that this stimulation was due to a microbial survival strategy in response to the limited resources, which led to the instability of the community. Furthermore, the observed slower temporal turnover of the bacterial community composition in response to eO_3 was due to the decreased deterministic processes derived from plants, which implied that eO_3 disrupted the coordination between soil microorganisms and rice crop. All above phenomena provided novel insights into the adverse influences of eO_3 on the soil microbial community. If O_3 concentration increases continuously, the adverse effects will be aggravated and harm the related ecological functions.

1. Introduction

Overall globally, managements lag behind the intensification of anthropogenic activities. Consequently, the concentration of ground-level ozone (O_3), one of the most significant phytotoxic gaseous pollutants, has been increasing since the Industrial Revolution (Vingarzan,

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2004). Unfortunately, it is expected to keep rising in the foreseeable future (Dentener et al., 2006). Such elevated O_3 (eO_3) is thus exerting a threat to ecosystems (Feng and Kobayashi, 2009; Li et al., 2017; Zak et al., 2011). Soil microorganisms, the drivers of matter, energy and signals in ecosystems, are sensitive to exogenous impacts. Thus, it allows us to use them as a proxy to study and predict the status of an ecosystem in response to environmental changes. The information about soil microorganisms will help us to better understand the response and the feedback of ecosystems to climate changes. However, compared to the numerous investigations on the aboveground information related to eO_3 (Feng et al., 2008; Li et al., 2017; Tang et al., 2015), information on underground microorganisms is still scarce and sometime inconsistent. Several reports have indicated that eO_3 adversely influenced soil microorganisms (Feng et al., 2013; Feng et al., 2011; Huang and Zhong, 2015; Zhang et al., 2016) due to the decrease in carbon flux from plants to soil and the alteration in nutrient conditions (Andersen, 2003; Biswas et al., 2008; Booker et al., 2005; Li et al., 2013). Specifically, eO_3 decreased the biomass and the diversity of fungi, methanotrophic bacteria and the groups involved in C and N fixation, as well as shifted their community compositions in wheat soils (Chieppa et al., 2015; Huang and Zhong, 2015; Li et al., 2013); for the aspect of microbial ecological functions, microbial C source utilization pattern was changed when exposed to eO_3 in rice (Chen et al., 2010) and wheat soils (Chen et al., 2009). However, the opposite findings have also been reported (Feng et al., 2015; Li et al., 2015). One of explanations for these superficially contradictory observations is that most of these studies were conducted at a single time point or within a short period. It is known that the influence of climate change is contingent on the duration of exposure time (Cheng et al., 2016; McKinley et al., 2009). Thus, a more reliable interpretation demands investigations across relative long-time scale. In addition, an insight into assembly mechanisms can help us to understand the reason why microbial community responds.

Unraveling the spatiotemporal variation in community composition and the generating mechanisms in response to environmental changes is an eternal theme in microbial ecology (Vellend, 2010). In addition, such a knowledge is the key to understand the response and feedback of ecosystems to climate change (Andersen, 2003). Ecological assembly (i.e. deterministic vs. stochastic) processes govern the turnover of community composition and are thought to be imperative in coupling with environmental changes (Ferrenberg et al., 2013; Graham et al., 2016; Graham et al., 2017; Graham and Stegen, 2017; Wang et al., 2013). In evaluating the distance-decay relationship (DDR) between community similarity and geographic distance, Deng et al. (2016) found that an elevated carbon dioxide (eCO_2) accelerated the spatial turnover (or heterogeneity) of soil microbial communities at the regional scale, due to increased stochastic processes derived from additional carbon resources. Nowadays, co-occurrence ecological network analysis is widely used to delineate the mechanism of potential biotic interactions within a community (Barberan et al., 2012; Dini-Andreote et al., 2014; Wood et al., 2017), beyond the aspects of composition and diversity (Faust and Raes, 2012). Furthermore, network analysis can offer insights into hub species and their shifts in responses to environmental changes (Deng et al., 2012; Feng et al., 2017a). Tu et al. (2016) and Yu et al. (2018) independently found that eCO_2 changed the co-occurrence network patterns and the hub species of nitrogen-fixing microbes. These consistent findings suggest a potential influence on the stability of microbial community and the efficiency of their ecological functions involved into soil nitrogen cycling. All these knowledge would contribute to our understanding of the influence of eO_3 on soil microorganisms and ecosystems. But obviously, the available information is still extremely scarce, far from sufficient for drawing meaningful and definitive conclusions.

In this study, with the China O_3 Free-Air Concentration Enrichment (O_3-FACE) platform established in 2007, we collected soil DNA samples (from 2009 to 2012) over a four-year period. Our goal is to verify temporal responses and assembly mechanisms of the soil bacterial community to eO_3. Specifically, we had three hypotheses in mind for this study to evaluate: (1) eO_3 decreases paddy soil bacterial diversity; (2) eO_3 leads to instability of the co-occurrence network pattern of the bacterial community; and (3) eO_3 influences the temporal turnover of the bacterial community composition and ecological assembly processes over four years. We believe that this information could provide novel insights into the understanding the responses of soil microorganisms to eO_3.

2. Materials and methods

2.1. China O_3-FACE system description

The O_3-FACE system was established in Jiangdu County, Jiangsu Province (119°45′E and 32°35′N) (Feng et al., 2011). The platform located in the subtropical climatic zone with a mean annual precipitation of 900–1000 mm, a mean annual temperature of 16 °C, an average daily integral radiation of 12.3 MJ m^{-2}, and a total of frost-free period of >230 days. The soil at the study site is classified as stagnic anthrosols according to Chinese soil taxonomy with a sandy-loamy texture of 9.2% of sand (1–0.05 mm), 65.7% of silt (0.05–0.001 mm), 25.1% of clay (<0.001 mm) and 1.2 g cm^{-3} of bulk density. The relevant soil chemical properties are 15.0 g kg^{-1} of soil organic C (SOC), 1.59 g kg^{-1} of total N, 1.23 g kg^{-1} of total P, 10.4 mg kg^{-1} of available P and pH 6.8.

The O_3-FACE project began on 1 July 2007. Briefly, this system has three plots exposed to elevated O_3 (hereinafter called eO_3) and other three plots under ambient O_3 (hereinafter called Ambient). Each plot had an area of approximately 240 m^2 and separated from each other at least 70 m to avoid cross-contamination. The target O_3 concentration in eO_3 was 50% higher than that in Ambient. Generally, the daily mean concentration of O_3 during rice cultivation was 38.2 ± 14.1 ppb for 2009, 42.4 ± 17.9 ppb for 2010, 35.3 ± 16.9 ppb for 2011 and 32.8 ± 13.5 ppb for 2012 in Ambient. Correspondingly, eO_3 had the values of 46.2 ± 21.4, 53.8 ± 27.4, 47.7 ± 27.1 and 43.1 ± 21.8 ppb. As for cultivated crops, summer rice (*Oryza sativa* L.) and winter wheat (*Triticum aestivum* L.) are managed in a rice-wheat rotation system. The detailed information on eO_3 system can refer to Tang et al. (2011), and that on rice-wheat rotation system had been fully described by Xu et al. (2006).

2.2. Sample collection and DNA extraction

72 rhizospheric soils (36 of eO_3 vs. 36 of Ambient) were sampled at the rice growth stages during 2009–2012. Each soil sample was based on combining rhizospheric soils from five rice plants, which were randomly taken in one plot at each time point. The detailed sampling information is listed in Table S1. Soil dissolved organic carbon (DOC) was extracted using 0.5 M K_2SO_4, and determined by TOC-TN analyzer (Skalar, Netherlands). Soil genomic DNA was extracted using FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA), dissolved in TE buffer, qualified by gel electrophoresis, and quantified by NanoDrop ND-2000 (ThermoFisher, USA). The extracted DNAs were stored at −20 °C until further use. In total, 36 DNA samples for Ambient and 36 DNA samples for eO_3 were collected in this investigation.

2.3. PCR and high-throughput sequencing of bacterial 16S rRNA genes

Bacterial 16S rRNA fragments were amplified using the primer set 519F/907R targeting hypervariable region V4–V5. The oligonucleotides of 5-bp barcode were fused to the forward primer to discriminate DNA samples. PCR reaction was carried out in a 50-μL reaction mixture and performed according to the following program: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. The triplicates of barcoded PCR products from each sample were purified using the QIAquick PCR Purification kit (Qiagen), then quantified using NanoDrop ND-2000, and finally pooled

together in equimolar amounts for pair-end sequencing on the Illumina MiSeq sequencing platform (Illumina Inc., CA, USA).

After sequencing was completed, the 16S rRNA genes data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010, http://qiime.source.org). Briefly, pair-end sequences were joined using FLASH and then assigned to each sample based on unique barcodes. After discarding those with quality score below 25 and a length fewer than 200 bp, qualified sequences were binned into operational taxonomic units (OTUs) using a 97% identity threshold, and the most abundant sequence from each OTU was selected as a representative sequence. In total, we obtained 2,298,450 sequences of qualified bacterial 16S rRNA gene with a minimum of 7077 per sample. Taxonomy was then assigned to OTUs using a subset of the SILVA 132 database (http://www.arb-silva.de/download/archive/qiime/) as a reference. All samples were then rarefied to 7000 sequences per sample to evaluate the α and β diversities of bacterial ecotypes. The obtained sequences in this study were deposited in the NCBI SRA database (accession no. SRP136673).

2.4. Diversity analysis

The observed OTUs, Shannon index, and phylogenetic diversity (PD) were calculated to evaluate the bacterial taxonomic richness and phylogenetic diversity in response to eO_3. Then the dissimilarities of the bacterial community composition between Ambient and eO_3 were calculated based on the Bray-Curtis distance, the unweighted Unifrac distance and the unweighted mean-nearest-taxon distance (βMNTD) (Fine and Kembel, 2011), which was visualized by nonmetric multidimensional scaling analyses (NMDS) and tested for significance by PERMANOVA. The rate of distance-decay of the bacterial community (β-diversity) was characterized by the slope of general linear regression between the change in temporal distance versus the bacterial composition similarity (Martiny et al., 2011). The similarity of the bacterial communities was calculated by a matrix of the pairwise Bray-Curtis distance among all samples from Ambient or eO_3. A permutation test was then conducted to test whether the distance-decay slope was significantly different between Ambient and eO_3. The above-mentioned analyses were implemented using SPSS 13.0 (SPSS Inc. Chicago, IL, USA) and the R packages vegan, ecodist and picante (version 3.0.2).

2.5. Calculation of estimated ecological processes

β-Nearest Taxon Index (βNTI) (Stegen et al., 2012) metrics was conducted to quantify the phylogenetic turnover between communities. The βNTI was estimated as the number of standard deviations that the observed βMNTD was from the mean of the null distribution of the βMNTD generated by 1000 randomizations of the 'phylogeny.pool' null model (Stegen et al., 2013). The relative contributions of deterministic processes (variable and homogeneous selections) were estimated as the percentage of pairwise βNTI values that fell above $+2$ and below -2, respectively (Stegen et al., 2015). To infer the relative influences of stochastic processes (homogenizing dispersal and dispersal limitation plus ecological drift), we combined the outcome of the βNTI analyses with a second null model referred to as a Bray-Curtis-based Raup-Crick (RC_{bray}), as described by Stegen et al. (2013). Then, if the pairwise $|\beta NTI| < 2$ but $RC_{bray} < -0.95$ or $> +0.95$, we infer that community turnover is governed by homogenizing dispersal or dispersal limitation plus ecological drift processes, respectively. Finally, in the case of $|\beta NTI| < 2$ and $|RC_{bray}| < 0.95$, no single process drives turnover in community composition.

2.6. Molecular ecological network analysis

Changes in phylogenetic molecular ecological networks (pMENs) between Ambient and eO_3 were evaluated using the random matrix theory (RMT)-based network approach (Luo et al., 2007). The pMEN construction and analyses were performed following the on-line pipeline (http://129.15.40.240/mena/) of Deng et al. (2012). The network graphs were then visualized using Gephi software. Network parameters, such as density, average centralization of degree, transitivity, average degree and average path distance etc., were used to evaluate the co-occurrence network topological structure within the community in response to eO_3. To decipher the importance of hub species, species were sorted into four subcategories: peripherals, connectors, module hubs, and network hubs based on the significance of connections among OTUs (Olesen et al., 2007).

2.7. Identification of bacterial species with time-discriminatory importance

To unravel the variations in the best discriminatory performance of taxa throughout the rice's life cycles between Ambient and eO_3, the relative abundances of bacterial taxa at the order level were regressed against four years using the function package 'randomForest' of R software with default parameters. The numbers of biomarker taxa were identified using 10-fold cross-validation that was implemented using R 'rfcv' function.

3. Results

3.1. Influence of eO_3 on the contents of DOC

The influence of eO_3 on the DOC contents is illustrated in Fig. 1 and Fig. S1. In general, eO_3 decreased DOC contents across the four-year period ($P < 0.05$). The decreasing pattern was observed for each of these four years, with a significant difference in 2012 ($P < 0.05$), marginal

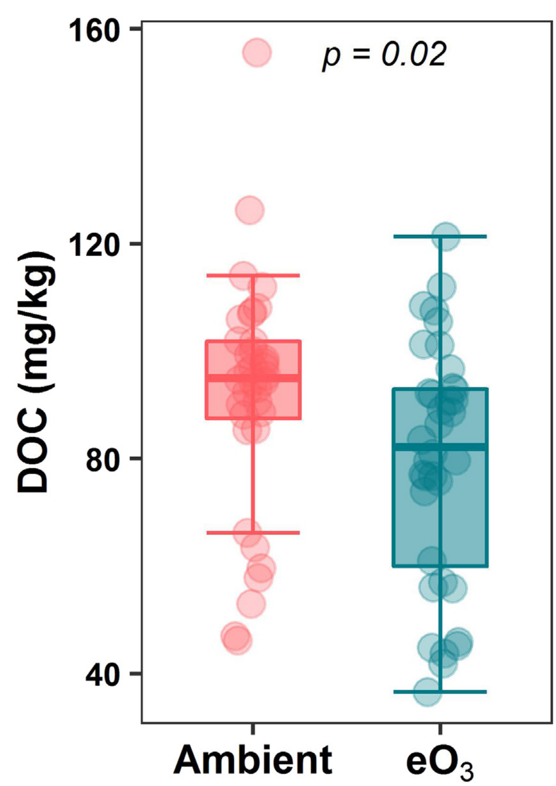

Fig. 1. The variations in the contents of dissolved organic carbon (DOC) between Ambient and elevated ground-level ozone (eO_3) across four years, i.e., from 2009 to 2012. Boxplot was drawn to depict DOC data through their quartiles. Five horizontal lines from top to bottom represent the largest value within 1.5 times interquartile range above the third quartile, the third quartile, the median, the first quartile and the smallest value within 1.5 times interquartile range below the first quartile, respectively. Comparison of means between treatments was done on Student's-*t*-test. Each treatment had 36 samples (that is, points). Ambient and eO_3 had daily average O_3 concentrations of 37.2 ± 4.14 ppb and 47.7 ± 4.49 ppb across four years, respectively.

differences in 2009 and 2010 ($0.05 < P < 0.1$) and no difference in 2011 ($P > 0.1$) (Fig. S1).

3.2. Influence of eO_3 on bacterial community diversities

The influence of eO_3 on the diversities of the paddy bacterial community is shown in Fig. 2 and Fig. S2. Contrary to our expectations, all alpha diversities increased under eO_3 ($P < 0.05$), regardless of the taxonomic and phylogenetic levels. However, we noticed that the majority of their values were not significant in each year (Fig. S2).

3.3. Influence of eO_3 on bacterial community compositions

The bacterial community composition also shifted in response to eO_3, indicated by both Bray-Curtis and unweighted Unifrac distance analyses (Fig. 3). The PERMANOVA test confirmed this conclusion ($P < 0.05$) (Table S2). Furthermore, we evaluated and compared the βMNTD distance-based bacterial community composition. As shown in Fig. 3 and Table S2, the phylogeny-based community composition also shifted significantly in response to eO_3 ($P < 0.05$). This result indicates that eO_3 influenced the composition of the bacterial community.

The plot of community similarity versus change in year shows universal negative slopes, indicating that when temporal distance increases, bacterial communities share less similarity (Fig. 4). The distance-decay slope is −0.014 for Ambient, while the value is −0.007 for eO_3. The pairwise comparison of slopes by the permutation tests indicated that the Ambient was steeper than the eO_3 ($P < 0.05$). This result suggests that eO_3 slowed the temporal compositional turnover of the bacterial community across the four years of rice cultivation.

3.4. Influence of eO_3 on patterns of ecological assembly processes governing temporal turnover

By estimating the null models of βNTI and RC_{bray}, we found that the ecological processes governing the community temporal turnover differed between Ambient and eO_3 (Fig. 5). Generally, homogeneous selection played an important role in the community turnover of bacterial taxa, with a contribution of 49.8% for Ambient and 45.4% for eO_3. The contribution of homogenizing dispersal increased from Ambient (12.9%) to eO_3 (19.0%). In contrast, the influences of differentiating processes (i.e., variable selection plus dispersal limitation) decreased, from 23.1% in Ambient to 19.7% in eO_3. Meanwhile, the deterministic processes (i.e., variable selection plus homogeneous selection) decreased from 51.5% in Ambient to 48.1% in eO_3.

3.5. The influence of eO_3 on patterns of bacterial co-occurrence network

Phylogenetic molecular ecological networks (pMENs) were generated to delineate the influence of eO_3 on the bacterial co-occurrence network (Fig. 6 and Table 1). Random networks were generated to test significant differences between the empirical and the simulated network indices ($P < 0.001$) (Table S3). Permutation tests indicated that all the empirical network indices were significantly different from the corresponding random counterpart (Table S3), and they were also different between Ambient and eO_3 ($P < 0.001$) (Table S4). Particularly, the values of density, degree of centralization, average degree and transitivity were statistically higher under eO_3 ($P < 0.001$). These findings consistently suggest that eO_3 complicated and centralized the bacterial co-occurrence network.

The topological role of each OTU in the networks was further determined based on its significance in connections with other OTUs (Fig. 7). According to the values of Zi and Pi, the roles of nodes were classified into four categories: peripherals, connectors, module hubs and network hubs. Peripherals represent specialists, while module hubs and connectors are closer to generalists, and network hubs are super generalists from an ecological perspective (Deng et al., 2012). A total of 4 nodes (accounting for 1.07% of the total nodes in the network) were connectors, and 12 nodes (2.36%) were module hubs under Ambient. In comparison, zero connectors and 8 module hubs (1.78%) were observed under eO_3 respectively.

3.6. Influence of eO_3 on bacterial species with time-discriminatory importance

To unravel the biomarker species discriminating the bacterial community compositions along the ages of rice cultivation, we performed

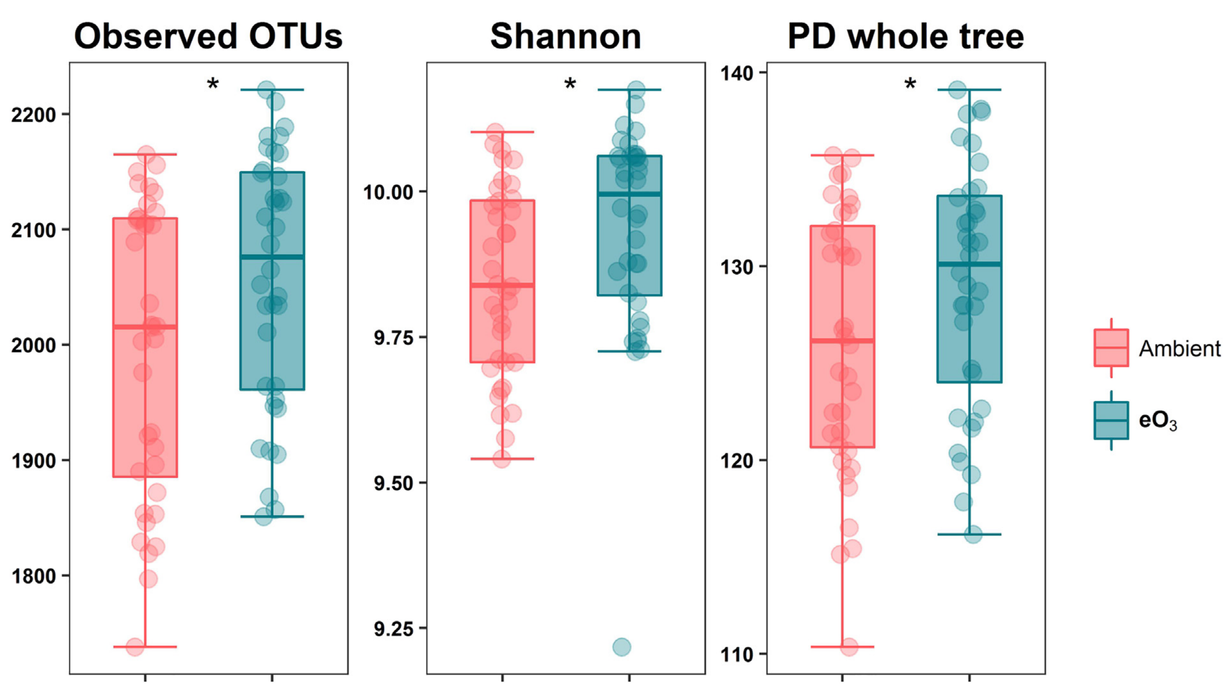

Fig. 2. The variations in the bacterial community diversities between Ambient and eO_3 across four years. OTUs and PD are the abbreviations of operational taxonomic units and phylogenetic diversity, respectively. Each treatment had 36 samples (that is, points).

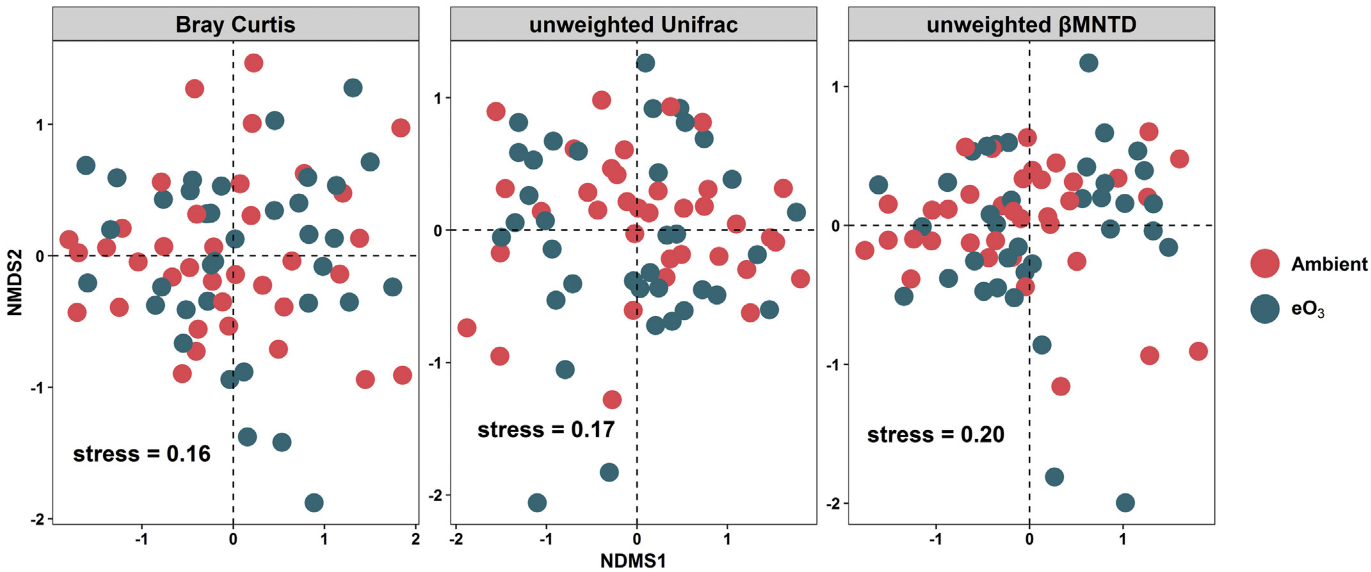

Fig. 3. The dissimilarities of the bacterial community composition between Ambient and eO_3 based on Bray-Curtis, unweighted Unifrac and unweighted between-community mean-nearest-taxon-distance (βMNTD) distances. Stress value of NMDS represents the difference between distance in the reduced dimension compared to the complete multidimensional space. The lower value indicates more reliability of NMDS ordination.

a regression of the relative abundances of bacterial species at the order level against four years of time, using the random forest machine learning algorithm. The minimum cross-validation error was obtained when using 53 and 36 important orders for Ambient and eO_3, respectively. The top 20 most important orders were chosen as the respective biomarker taxa because cross-validation error curve had stabilized when using these orders for both Ambient and eO_3 (Fig. 8). Pairwise comparison indicated that the most important biomarker species for the time discrimination shifted from Bacteroidetes under Ambient to Actinobacteria under eO_3. Meanwhile, the heat-map plot revealed that the relative abundance of Actinobacteria increased with rice cultivation age under eO_3.

4. Discussion

4.1. eO_3 increases alpha diversity of bacterial community

Paddy fields play important roles in global crop yields and the biogeochemical cycling of elements (Liesack et al., 2000). An accurate

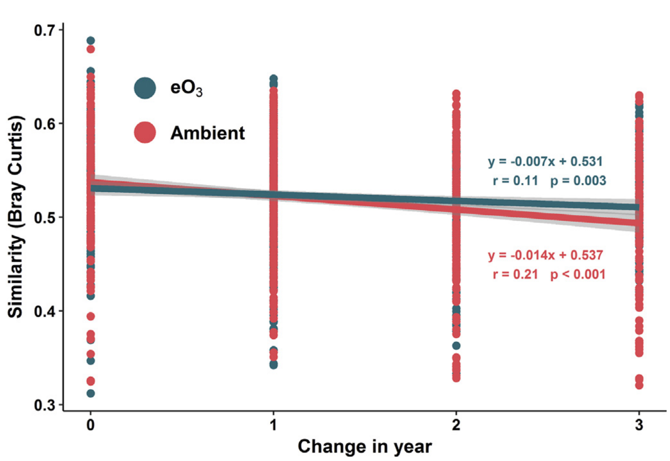

Fig. 4. Distance-decay curves of soil bacterial community under Ambient and eO_3 across four years (i.e., 2009–2012) ($n = 630$). The y-axis is the microbial community similarity calculated using the Bray-Curtis distance. The x-axis is the temporal distance in year. The slopes were pairwise compared between Ambient and eO_3, and they were significantly different ($P < 0.05$).

interpretation of paddy microbial responses, together with an insight into the underlying assembly mechanisms influenced by O_3 pollution, will significantly contribute to the understanding of comprehensive scenario of the responses and feedback of paddy ecosystems. We previously found that eO_3 negatively influenced the microbial community in paddy soils (Feng et al., 2013; Feng et al., 2011; Zhang et al., 2016). However, it was unexpected to find that eO_3 increased their alpha diversities in this study (Fig. 2). This information is opposite to our first hypothesis, and the result seems to oppose a common consensus that eO_3 has adverse influences on soil microorganisms. Indeed, our finding is not unique; similar results have been reported previously (Feng et al., 2015; Li et al., 2015). As described by Hutchinson (1965) in "The ecological theater and the evolutionary play", the evolutionary viewpoint could give us an informative explanation. The Black Queen Hypothesis posits that in an oligotrophic environment, adaptive genome reduction can provide a selective advantage by conserving a microorganism's limiting resources (Mas et al., 2016; Morris et al., 2012). In this situation, microbial species become cross-feeding relationship and tend to mutualistic symbiosis for survival as the strategy to handle limited resources (Pande and Kost, 2017). Consequently, their diversity increased, which is observed in the investigation by Großkopf and Soyer (2016) from the perspective of the first principles of thermodynamics. Consistently, we found a highly densified co-occurrence network under the eO_3 condition in comparison with that under the Ambient condition (Table 1 and Fig. 6). The increased diversity could be attributed to lower carbon input from plant into soils in response to eO_3. It is well known that plants are the primary carbon source for soil microorganism; O_3 enters leafs through stomata, induces oxidative stress in crop cells, and inhibits photosynthesis and physiological processes. Collectively, they lead to retarded crop growth and reduced biomass, and in the end, decreased the carbon flux from plant into soils (Akhtar et al., 2010; Tang et al., 2015). It is exemplified that decreased DOC contents were observed under eO_3 (Fig. 1). A similar phenomenon was observed by our previous work, i.e., the arable soils without nutrient additions had higher bacterial diversity than soils with added nutrients (Feng et al., 2017b). Another explanation on this observed discrepancy could be due to experiment itself. Most of those studies were conducted at a single time point or within a short period. It is well known that the influence of climate change on an ecosystem is contingent on the duration of the exposure time. For instance, the gross rates of N mineralization in a woodland, which is driven by soil microorganisms, presented

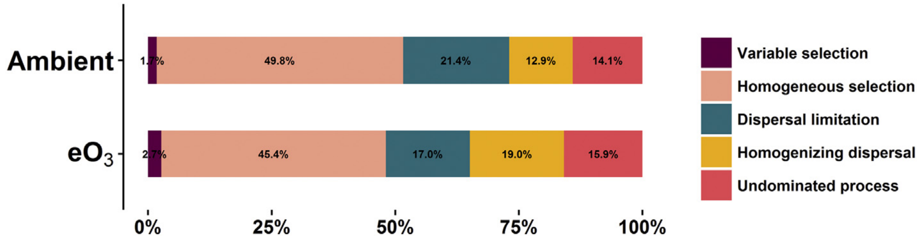

Fig. 5. Contributions of ecological processes governing bacterial community turnover under Ambient and eO_3, respectively, across four years (i.e., 2009–2012).

divergent responses to eCO_2, with reductions after six years but a slight increase after eleven years (McKinley et al., 2009). Therefore, our results from the long-term study could produce different results from previous ones.

4.2. eO_3 leads to high centralization of co-occurrence of bacterial community

The centralization of the co-occurrence network was observed on eO_3 (Table 1 and Fig. 6). As the streamlining theory predicts, an increase in the competition for resources is always concurrent with increases in cell-cell dependency among microbial members (Giovannoni et al., 2014). A highly centralized microbial community is sensitive to external disturbances because cascading collapses of the interspecies network can be easily triggered. Changes in transitivity, average harmonic geodesic distances, as well as geodesic efficiencies, support the above prediction (Table 1). A higher transitivity indicates stronger dependency and couplings within the community (Narisawa et al., 2008), and perils the stability of the co-occurrence network (Wood et al., 2017). Similarly, smaller average harmonic geodesic distances and the larger geodesic efficiencies of the eO_3 network indicate that all nodes were closer, and the interspecies dependencies were higher (Latora and Marchiori, 2001; West, 1996). Furthermore, it was found that the numbers of hub species in modules (module hubs) and between modules (connectors) decreased in response to eO_3 (Fig. 7). As suggested by Deng et al. (2012), module hubs are species that are highly connected to several other species in their own modules, while connectors are species that are highly linked to multiple modules. Since each network module represents a specific potential ecological function (Deng et al., 2012; Ling et al., 2016), a decrease in hub species implies a possible destabilization of the community and, consequently, a low efficiency of the microbial ecological functions (Feng et al., 2017b). Collectively, we postulated that the high centralization and the decreased hub species in eO_3 destabilize the paddy ecosystems and harm the ecological functions they provided.

4.3. eO_3 slows the temporal turnover of bacterial community composition

It is known that there are distinct successional dynamics in microbial community composition throughout the life cycle of rice (Edwards et al., 2018; Zhang et al., 2018). Namely, there could be a temporal coordination or an intimate interaction between soil microorganisms and rice host. Soil microorganisms act as the indispensable underground constituent of the plant system, in which they obtain energy and resources from plants (Kiers et al., 2011). The deterministic influence of a plant was thus imposed on soil microbial community assembly (Feng et al., 2017b). This result was corroborated by the evidence of the deterministic topological structures of the co-occurrence networks, rather than the stochastic (Table S3) and predominant influences of homogeneous selection on assembly processes (Fig. 5). Thus, a clear temporal DDR correlation was observed throughout the life cycle of rice (Fig. 4). Under eO_3, the temporal turnover of the bacterial community

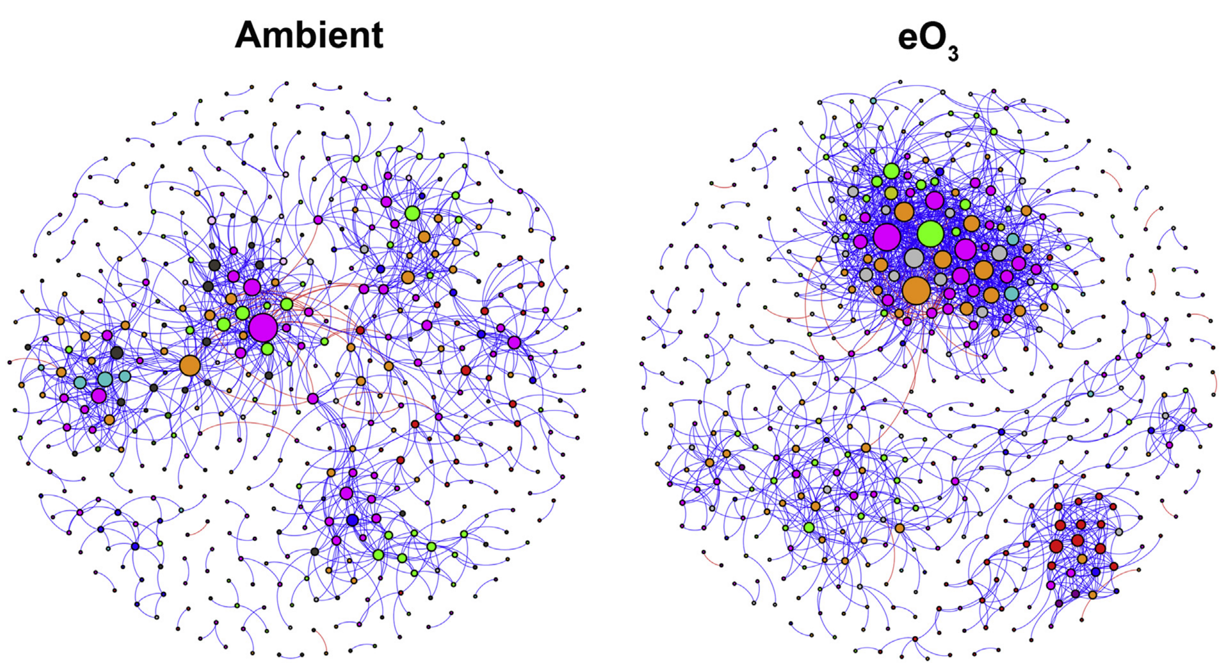

Fig. 6. The visualization of the bacterial network associations in Ambient and eO_3 based on random matrix theory (RMT) analysis from OTU profiles. Red and blue lines respectively represent negative and position correlations between nodes. The size of each node is proportional to the number of connections (that is, degree).

Table 1
Topological properties of bacterial molecular ecological networks between Ambient and eO_3.

Topological properties	Ambient	eO_3
Total nodes	467	505
Total links	1072	1628
R square of power-law	0.906	0.906
Average degree (avgK)	4.591	6.448
Average clustering coefficient (avgCC)	0.285	0.327
Average path distance (GD)	4.980	5.259
Geodesic efficiency (E)	0.240	0.254
Module	40	52
Modularity	0.72	0.591
Transitivity (Trans)	0.305	0.377
Harmonic geodesic distance (HD)	4.161	3.932
Maximal degree	49	64
Centralization of degree (CD)	0.096	0.115
Maximal betweenness	24,568	29,740
Centralization of betweenness (CB)	0.222	0.231
Maximal stress centrality	145,569	260,515
Centralization of stress centrality (CS)	1.307	2.016
Maximal eigenvector centrality	0.388	0.260
Centralization of eigenvector centrality (CE)	0.370	0.243
Density (D)	0.010	0.013
Connectedness (Con)	0.657	0.517
Efficiency	0.988	0.979

composition decreased compared to that of Ambient and had a lower slope value. Consistently, the influences of temporal differentiating processes decreased under eO_3 (19.7%) compared to Ambient (23.1%) (Fig. 5). The reason is speculated to be a result of the decrease in the deterministic process of assembly mechanism (Fig. 5), which is consistent with other findings (Barberan and Casamayor, 2010; Hewson et al., 2006). Hence, our third hypothesis was confirmed. There are several possible explanations for this result. First, eO_3 adversely influences plant growth and decreases root exudates (Li et al., 2017; Wang et al., 2016). The root exudates are the key substances linking soil microorganisms with plants (Maillet et al., 2011). Naturally, their decreases will weaken the coordination between microorganisms and plants. Second, the decrease in soil C inputs under eO_3 may drive microbial species from being copiotroph-dominant with high growth rates, to oligotroph-dominant with slow growth rates. As evidence to this postulation, we observed that the species in the phylum Actinobacteria became dominant and contributed significantly to the variations in the bacterial community composition under eO_3 relative to those in the phylum Bacteroidetes under Ambient (Fig. 8). The phyla Bacteroidetes and Actinobacteria can be classified into the *r*- and the *K*-selected categories, respectively, which correspond to the copiotrophic and the oligotrophic (Fierer et al., 2007). Third, the decreased substrates in soil, partially exemplified by decreased DOC contents (Fig. 1), stretches the generation time (e.g., fewer generations in a given time), and changes the microbial composition. Taken all together, lower temporal compositional turnover was observed in response to eO_3. This result further implies that eO_3 had disrupted the coordination between soil microorganisms and the rice system. With an accumulation of eO_3, the effect of disruption is strengthened and, in turn, eventually harms rice growth and the paddy ecosystem.

4.4. Implication of distinctly responding microorganisms to paddy ecosystem in response to eO_3

The Actinobacteria species were observed to distinctly respond to eO_3 and play important roles in shifting the community composition across the four years (Fig. 8). Among them, members of phylum Actinobacteria are abundant in soils. They are essential for the degradation of organic matter, especially recalcitrant substances such as cellulose and polycyclic aromatic hydrocarbons (de Menezes et al., 2015; Lewin et al., 2016; Sagova-Mareckova et al., 2011). Therefore, the increased importance of Actinobacteria under eO_3 implies that, while carbon flux from the plant decreases, the soil microbial community compensates the deficit by degrading recalcitrant organic matters,

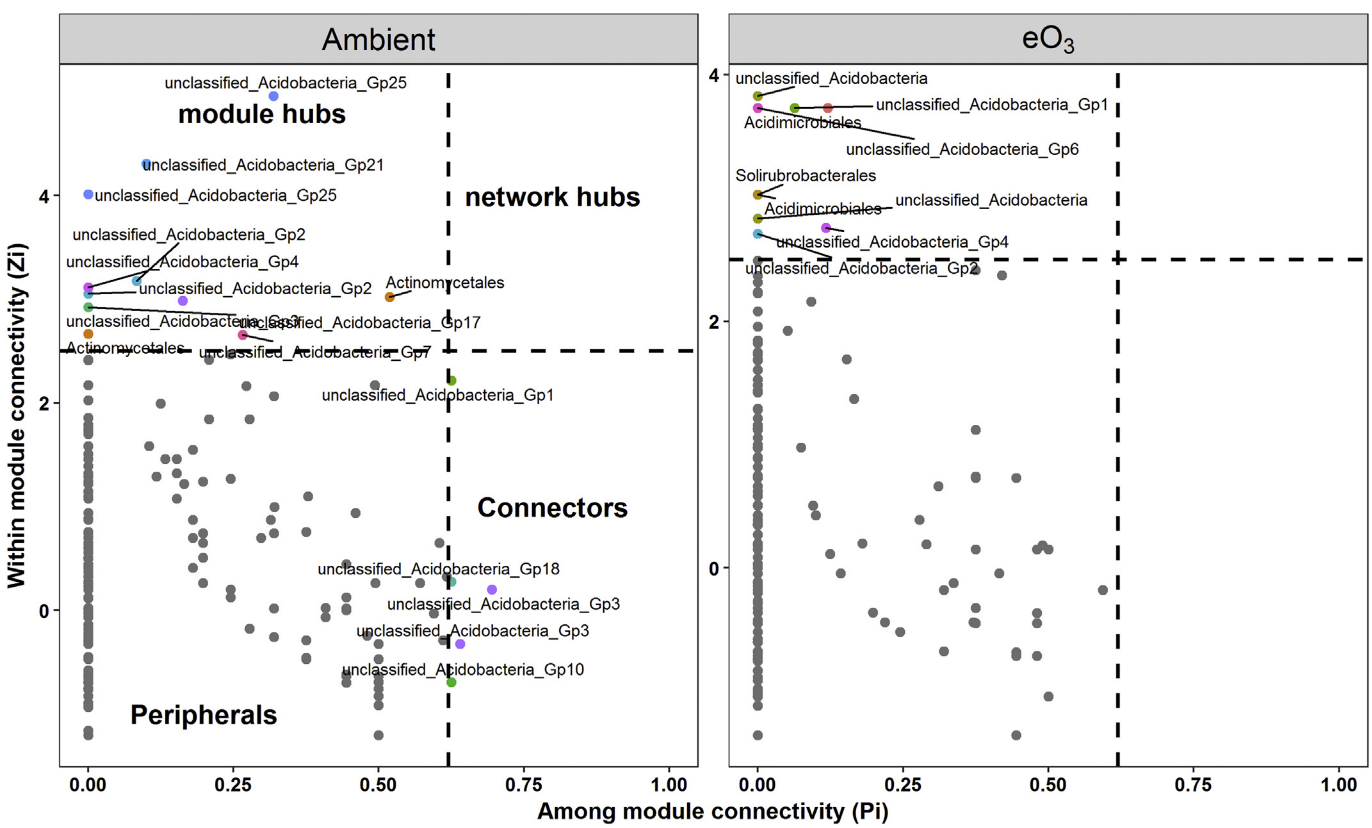

Fig. 7. Zi-Pi plot showing the distribution of OTUs based on their topological roles from soil samples under Ambient and eO_3.

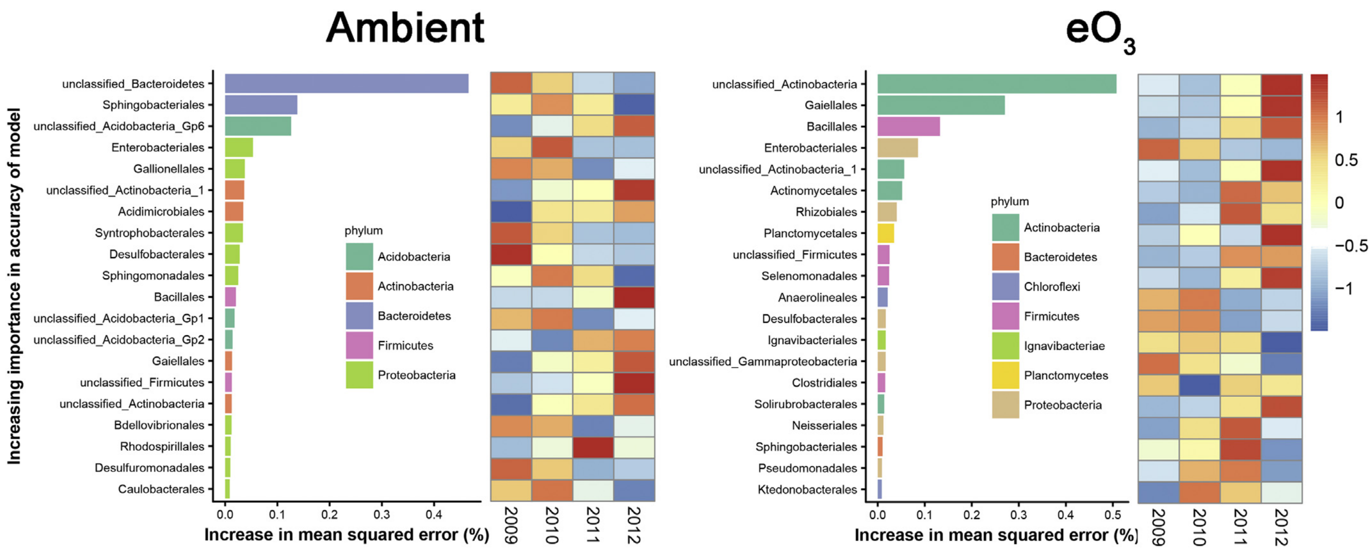

Fig. 8. Bacterial taxonomic biomarkers of four-year rice cultivation under Ambient and eO_3, respectively. The top 20 biomarker taxa were ranked in descending order of time-discriminatory importance to the accuracy of the models. Heatmap showed the variations in the relative abundances of the top 20 predictive biomarker taxa across four years.

such as soil organic carbon (SOC) to obtain the necessary energy and carbon sources for growth. In this situation, much of SOC would be consumed. Unfortunately, SOC is the major index of soil fertility and sustainability of arable lands (Gregorich et al., 1994; Jimenez et al., 2002). Thus, a continuous increase in this kind of microbes under long-term eO_3 will be detrimental to soil fertility and sustainability.

5. Conclusion

In this investigation, we presented the four-year temporal responses of a paddy bacterial community to eO_3, proposed the underlying assembly mechanisms and predicted the possible feedbacks to the paddy ecosystem in the future. Contrary to commonly believed, we found that bacterial alpha diversity increased under eO_3. However, this increase was the result of survival strategy adopted by the microbial in response to limited resources, which was posited by the Black Queen Hypothesis and the streamlining theory. For the same reason, the high centralization of the co-occurrence network was observed in eO_3 as well as the slower temporal turnover of the community composition, implying an instability in the bacterial community and a disrupted coordination between soil microorganisms and rice crop. All findings suggested the adverse influence of eO_3 on the soil bacterial community and the possible negative feedback to the paddy ecosystem, caused by continuous increase in O_3. In addition, our result suggested that, besides bacteria, fungi and some functional microbial groups could also be interesting subjects for future researches. Meantime, our information highlights a need to strengthen the theoretical linkages between microbial ecology and climate change at large spatial and temporal scales.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.11.130.

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